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I, KIM MARSHALL, MANAGER EXAMINATION SUPPORT AND SALES, hereby certify that the annexed is a true copy of the Provisional specification in connection with Application No. PO 6973 for a patent by THE COUNCIL OF THE QUEENSLAND INSTITUTE OF MEDICAL RESEARCH filed on 23 May 1997.

I further certify that the annexed specification is not, as yet, open to public inspection.

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day of June 1998

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Regulation 3.2

The Council of The Queensland Institute of Medical Research

A U S T R A L I A

Patents Act 1990

PROVISIONAL SPECIFICATION

for the invention entitled:

"A novel gene and uses therefor"

The invention is described in the following statement:

A NOVEL GENE AND USES THEREFOR

The present invention relates generally to a novel human gene and to derivatives and mammalian, animal, insect, nematode, avian and microbial homologues thereof. The present invention further provides pharmaceutical compositions and diagnostic agents as well as genetic molecules useful in gene replacement therapy and recombinant molecules useful in protein replacement therapy.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers.

Sequence Identity Numbers (SEQ ID NOs.) for the nucleotide and amino acid sequences referred to in the specification are defined at the end of the description.

The increasing sophistication of recombinant DNA technology is greatly facilitating research and development in the medical and allied health fields. There is growing need to develop recombinant and genetic molecules for use in diagnosis, conventional pharmaceutical preparations as well as gene and protein replacement therapies.

In work leading up to the present invention, the inventors sought to identify and clone human genes which might be useful as potential diagnostic and/or therapeutic agents. On area of particular interest is in the field of heat shock proteins. The *Escherichia coli* heat shock protein DnaJ is the founding member of a family of proteins that are associated with protein folding, protein complex assembly and transit through subcellular components.

Prokaryotic and eukaryotic DnaJ homologues have a modular organisation consisting of a J

domain, a glycine-rich spacer, CXXCXGXG repeats and a C-terminal region with no obvious sequence features, as well as additional sequences for protein targeting. The J domain is anticipated to mediate interaction with heat shock 70 proteins (Hsp70) and consists of some 70 amino acids, frequently located at the N-terminus of the protein.

In accordance with the present invention, the inventors have identified a novel gene on chromosome 11q13 which encodes a protein. It is proposed that the protein is a heat shock protein and may have a role in tumour suppression.

Accordingly, one aspect of the present invention contemplates an isolated nucleic acid molecule comprising a sequence of nucleotides encoding or complementary to a sequence encoding an amino acid sequence having homology to a heat shock protein or a derivative of said heat shock protein or a heat shock-binding protein.

More particularly, the present invention is directed to an isolated nucleic acid molecule comprising a sequence of nucleotides or a complementary form thereof selected from:

(i) a nucleotide sequence set forth in SEQ ID NO:1;

(ii) a nucleotide sequence encoding an amino acid sequence set forth in SEQ ID NO:2;

(iii) a nucleotide sequence having at least about 40% similarity to the nucleotide sequence of (i) or (ii); and

(iv) a nucleotide sequence capable of hybridizing under low stringency conditions to the nucleotide sequence set forth in (i), (ii) or (iii).

Preferably, the percentage similarity is at least about 50%. More preferably, the percentage similarity is at least about 60%.

Reference herein to a low stringency at 42°C includes and encompasses from at least about 1% v/v to at least about 15% v/v formamide and from at least about 1M to at least about 2M salt for hybridisation, and at least about 1M to at least about 2M salt for washing conditions. Alternative

stringency conditions may be applied where necessary, such as medium stringency, which includes and encompasses from at least about 16% v/v to at least about 30% v/v formamide and from at least about 0.5M to at least about 0.9M salt for hybridisation, and at least about 0.5M to at least about 0.9M salt for washing conditions, or high stringency, which includes and encompasses from at least about 31% v/v to at least about 50% v/v formamide and from at least about 0.01M to at least about 0.15M salt for hybridisation, and at least about 0.01M to at least about 0.15M salt for washing conditions.

The term "similarity" as used herein includes exact identity between compared sequences at the nucleotide or amino acid level. Where there is non-identity at the nucleotide level, "similarity" includes differences between sequences which result in different amino acids that are nevertheless related to each other at the structural, functional, biochemical and/or conformational levels. Where there is non-identity at the amino acid level, "similarity" includes amino acids that are nevertheless related to each other at the structural, functional, biochemical and/or conformational levels.

The present invention extends to nucleic acid molecules with percentage similarities of approximately 65%, 70%, 75%, 80%, 85%, 90% or 95% or above or a percentage in between.

The nucleic acid molecule of the present invention is hereinafter referred to as constituting the "*mcg18*" gene. The protein encoded by *mcg18* is referred to herein as "MCG18".

The present invention extends to the naturally occurring genomic *mcg18* nucleotide sequence or corresponding cDNA sequence or to derivatives thereof. Derivatives contemplated in the present invention include fragments, parts, portions, mutants, homologues and analogues of MCG18 or the corresponding genetic sequence. Derivatives also include single or multiple amino acid substitutions, deletions and/or additions to MCG18 or single or multiple nucleotide substitutions, deletions and/or additions to *mcg18*. "Additions" to the amino acid or nucleotide sequences include fusions with other peptides, polypeptides or proteins or fusions to nucleotide sequences. Reference herein to "MCG18" or "*mcg18*" includes references to all derivatives thereof including functional derivatives and immunologically

interactive derivatives of MCG18.

The *mcg18* of the present invention is particularly exemplified herein from humans and in particular from human chromosome 11q13.

The present invention extends, however, to a range of homologues from, for example, primates, livestock animals (eg. sheep, cows, horses, donkeys, pigs), companion animals (eg. dogs, cats) laboratory test animals (eg. rabbits, mice, rats, guinea pigs), birds (eg. chickens, ducks, geese, parrot), insects, nematodes, eukaryotic microorganism and captive wild animals (eg. deer, foxes, kangaroos). Reference herein to *mcg18* or MCG18 includes reference to these molecules of human origin as well as novel forms of non-human origin.

The nucleic acid molecules of the present invention may be DNA or RNA. When the nucleic acid molecule is in DNA form, it may be genomic DNA or cDNA. RNA forms of the nucleic acid molecules of the present invention are generally mRNA.

Although the nucleic acid molecules of the present invention are generally in isolated form, they may be integrated into or ligated to or otherwise fused or associated with other genetic molecules such as vector molecules and in particular expression vector molecules. Vectors and expression vectors are generally capable of replication and, if applicable, expression in one or both of a prokaryotic cell or a eukaryotic cell. Preferably, prokaryotic cells include *E. coli*, *Bacillus sp* and *Pseudomonas sp*. Preferred eukaryotic cells include yeast, fungal, mammalian and insect cells.

Accordingly, another aspect of the present invention contemplates a genetic construct comprising a vector portion and an animal, more particularly a mammalian and even more particularly a human *mcg18* gene portion, which *mcg18* gene portion is capable of encoding an MCG18 polypeptide or a functional or immunologically interactive derivative thereof.

Preferably, the *mcg18* gene portion of the genetic construct is operably linked to a promoter on the vector such that said promoter is capable of directing expression of said *mcg18* gene portion

in an appropriate cell.

In addition, the *mcg18* gene portion of the genetic construct may comprise all or part of the gene fused to another genetic sequence such as a nucleotide sequence encoding glutathione-S-transferase or part thereof.

The present invention extends to such genetic constructs and to prokaryotic or eukaryotic cells comprising same.

It is proposed in accordance with the present invention that MCG18 is a transcription factor involved in protein folding, protein complex assembly and transit through subcellular compartments. MCG18 may also have a role in tumour suppression. Thus mutations in *mcg18* may result in the development of or a propensity to develop various types of cancer.

A deletion or aberration in the *mcg18* gene may also be important in the detection of cancer or a propensity to develop cancer. An aberration may be a homozygous mutation or a heterozygous mutation. The detection may occur at the foetal or post-natal level. Detection may also be at the germline or somatic cell level. Furthermore, a risk of developing cancer may be determined by assaying for aberrations in the parents and/or proband of the subject under investigation.

According to this aspect of the present invention, there is contemplated a method of detecting a condition caused or facilitated by an aberration in *mcg18,* said method comprising determining the presence of a single or multiple nucleotide substitution, deletion and/or addition or other aberration to one or both alleles of said *mcg18* wherein the presence of such a nucleotide substitution, deletion and/or addition or other aberration may be indicative of said condition or a propensity to develop said condition.

The nucleotide substitutions, additions or deletions may be detected by any convenient means including nucleotide sequencing, restriction fragment length polymorphism (RFLP), polymerase chain reaction (PCR), oligonucleotide hybridization and single stranded

conformation polymorphism analysis (SSCP) amongst many others. An aberration includes modifications to existing nucleotides such as to modify glycosylation signals amongst other effects.

In an alternative method, aberrations in the *mcg18* gene are detected by screening for mutations in MCG18.

A mutation in MCG18 may be a single or multiple amino acid substitution, addition and/or deletion. The mutation in *mcg18* may also result in either no translation product being produced or a product in truncated form. A mutation may also be an altered glycosylation pattern or the introduction of side chain modifications to amino acid residues.

According to this aspect of the present invention, there is provided a method of detecting a condition caused or facilitated by an aberration in *mcg18,* said method comprising screening for a single or multiple amino acid substitution, deletion and/or addition to MCG18 wherein the presence of such a mutation is indicative of or a propensity to develop said condition.

A particularly convenient means of detecting a mutation in MCG18 is by use of antibodies.

Accordingly another aspect of the present invention is directed to antibodies to MCG18 and its derivatives. Such antibodies may be monoclonal or polyclonal and may be selected from naturally occurring antibodies to MCG18 or may be specifically raised to MCG18 or derivatives thereof. In the case of the latter, MCG18 or its derivatives may first need to be associated with a carrier molecule. The antibodies to MCG18 of the present invention are particularly useful as diagnostic agents.

For example, antibodies to MCG18 and its derivatives can be used to screen for wild-type MCG18 or for mutated MCG18 molecules. The latter may occur, for example, during or prior to certain cancer development. A differential binding assay is also particularly useful. Techniques for such assays are well known in the art and include, for example, sandwich assays and ELISA. Knowledge of normal MCG18 levels or the presence of wild-type MCG18 may be

important for diagnosis of certain cancers or a predisposition for development of cancers or for monitoring certain therapeutic protocols.

As stated above antibodies to MCG18 of the present invention may be monoclonal or polyclonal or may be fragments of antibodies such as Fab fragments. Furthermore, the present invention extends to recombinant and synthetic antibodies and to antibody hybrids. A "synthetic antibody" is considered herein to include fragments and hybrids of antibodies.

For example, specific antibodies can be used to screen for wild-type MCG18 molecule or specific mutant molecules such as molecules having a certain deletion. This would be important, for example, as a means for screening for levels of MCG18 in a cell extract or other biological fluid or purifying MCG18 made by recombinant means from culture supernatant fluid or purified from a cell extract. Techniques for the assays contemplated herein are known in the art and include, for example, sandwich assays and ELISA.

It is within the scope of this invention to include any second antibodies (monoclonal, polyclonal or fragments of antibodies or synthetic antibodies) directed to the first mentioned antibodies discussed above. Both the first and second antibodies may be used in detection assays or a first antibody may be used with a commercially available anti-immunoglobulin antibody. An antibody as contemplated herein includes any antibody specific to any region of wild-type MCG4 or to a specific mutant phenotype or to a deleted or otherwise altered region.

Both polyclonal and monoclonal antibodies are obtainable by immunization of a suitable animal or bird with MCG18 or its derivatives and either type is utilizable for immunoassays. The methods of obtaining both types of sera are well known in the art. Polyclonal sera are less preferred but are relatively easily prepared by injection of a suitable laboratory animal or bird with an effective amount of MCG18 or antigenic parts thereof or derivatives thereof, collecting serum from the animal or bird, and isolating specific sera by any of the known immunoadsorbent techniques. Although antibodies produced by this method are utilizable in virtually any type of immunoassay, they are generally less favoured because of the potential heterogeneity of the product.

The use of monoclonal antibodies in an immunoassay is particularly preferred because of the ability to produce them in large quantities and the homogeneity of the product. The preparation of hybridoma cell lines for monoclonal antibody production derived by fusing an immortal cell line and lymphocytes sensitized against the immunogenic preparation can be done by techniques which are well known to those who are skilled in the art.

Another aspect of the present invention contemplates a method for detecting MCG18 or a derivative thereof in a biological sample said method comprising contacting said biological sample with an antibody specific for MCG18 or its derivatives or homologues for a time and under conditions sufficient for an antibody-MCG18 complex to form, and then detecting said complex.

Preferably, the biological sample is a cell extract from a human or other animal or a bird.

The presence of MCG18 may be accomplished in a number of ways such as by Western blotting and ELISA procedures. A wide range of immunoassay techniques are available as can be seen by reference to US Patent Nos. 4,016,043, 4, 424,279 and 4,018,653. These includes both single-site and two-site or "sandwich" assays of the non-competitive types, as well as in traditional competitive binding assays. These assays also include direct binding of a labelled antibody to a target.

Sandwich assays are among the most useful and commonly used assays and are favoured for use in the present invention. A number of variations of the sandwich assay technique exist, and all are intended to be encompassed by the present invention. Briefly, in a typical forward assay, an unlabelled antibody is immobilized on a solid substrate and the sample to be tested brought into contact with the bound molecule. After a suitable period of incubation, for a period of time sufficient to allow formation of an antibody-antigen complex, a second antibody specific to the antigen, labelled with a reporter molecule capable of producing a detectable signal is then added and incubated, allowing time sufficient for the formation of another complex of antibody-antigen-labelled antibody. Any unreacted material is washed away, and the presence of the antigen is determined by observation of a signal produced by the reporter molecule. The results may either

be qualitative, by simple observation of the visible signal, or may be quantitated by comparing with a control sample containing known amounts of hapten. Variations on the forward assay include a simultaneous assay, in which both sample and labelled antibody are added simultaneously to the bound antibody. These techniques are well known to those skilled in the art, including any minor variations as will be readily apparent. In accordance with the present invention the sample is one which might contain MCG18 including cell extract or, tissue biopsy. The sample is, therefore, generally a biological sample comprising biological fluid but also extends to fermentation fluid and supernatant fluid such as from a cell culture.

In the typical forward sandwich assay, a first antibody having specificity for the MCG18 or an antigenic part thereof or a derivative thereof or antigenic parts thereof, is either covalently or passively bound to a solid surface. The solid surface is typically glass or a polymer, the most commonly used polymers being cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene. The solid supports may be in the form of tubes, beads, discs of microplates, or any other surface suitable for conducting an immunoassay. The binding processes are well-known in the art and generally consist of cross-linking covalently binding or physically adsorbing, the polymer-antibody complex is washed in preparation for the test sample. An aliquot of the sample to be tested is then added to the solid phase complex and incubated for a period of time sufficient (e.g. 2-40 minutes) and under suitable conditions (e.g. 25°C) to allow binding of any subunit present in the antibody. Following the incubation period, the antibody subunit solid phase is washed and dried and incubated with a second antibody specific for a portion of the hapten. The second antibody is linked to a reporter molecule which is used to indicate the binding of the second antibody to the hapten.

An alternative method involves immobilizing the target molecules in the biological sample and then exposing the immobilized target to specific antibody which may or may not be labelled with a reporter molecule. Depending on the amount of target and the strength of the reporter molecule signal, a bound target may be detectable by direct labelling with the antibody. Alternatively, a second labelled antibody, specific to the first antibody is exposed to the target-first antibody complex to form a target-first antibody-second antibody tertiary complex. The complex is detected by the signal emitted by the reporter molecule.

By "reporter molecule" as used in the present specification, is meant a molecule which, by its chemical nature, provides an analytically identifiable signal which allows the detection of antigen-bound antibody. Detection may be either qualitative or quantitative. The most commonly used reporter molecules in this type of assay are either enzymes, fluorophores or radionuclide containing molecules (i.e. radioisotopes) and chemiluminescent molecules.

In the case of an enzyme immunoassay, an enzyme is conjugated to the second antibody, generally by means of glutaraldehyde or periodate. As will be readily recognized, however, a wide variety of different conjugation techniques exist, which are readily available to the skilled artisan. Commonly used enzymes include horseradish peroxidase, glucose oxidase, beta-galactosidase and alkaline phosphatase, amongst others. The substrates to be used with the specific enzymes are generally chosen for the production, upon hydrolysis by the corresponding enzyme, of a detectable colour change. Examples of suitable enzymes include alkaline phosphatase and peroxidase. It is also possible to employ fluorogenic substrates, which yield a fluorescent product rather than the chromogenic substrates noted above. In all cases, the enzyme-labelled antibody is added to the first antibody hapten complex, allowed to bind, and then the excess reagent is washed away. A solution containing the appropriate substrate is then added to the complex of antibody-antigen-antibody. The substrate will react with the enzyme linked to the second antibody, giving a qualitative visual signal, which may be further quantitated, usually spectrophotometrically, to give an indication of the amount of hapten which was present in the sample. "Reporter molecule" also extends to use of cell agglutination or inhibition of agglutination such as red blood cells on latex beads, and the like.

Alternately, fluorescent compounds, such as fluorescein and rhodamine, may be chemically coupled to antibodies without altering their binding capacity. When activated by illumination with light of a particular wavelength, the fluorochrome-labelled antibody adsorbs the light energy, inducing a state to excitability in the molecule, followed by emission of the light at a characteristic colour visually detectable with a light microscope. As in the EIA, the fluorescent labelled antibody is allowed to bind to the first antibody-hapten complex. After washing off the unbound reagent, the remaining tertiary complex is then exposed to the light of the appropriate wavelength the fluorescence observed indicates the presence of the hapten of interest. Immunofluorescence and EIA techniques are both very well established in the art and are

particularly preferred for the present method. However, other reporter molecules, such as radioisotope, chemiluminescent or bioluminescent molecules, may also be employed.

As stated above, MCG18 is proposed to have a role in tumour suppression. Accordingly, it is further proposed in accordance with the present invention to use recombinant MCG18 in pharmaceutical preparations for treating arresting or otherwise ameliorating the effects of certain cancers.

Accordingly, another aspect of the present invention contemplates a method for treating, arresting or otherwise ameliorating the effects of a cancer in an animal or bird, said method comprising administering to said animal or bird an effective amount of MCG18 or a functional derivative thereof for a time and under conditions sufficient to treat, arrest or otherwise ameliorate the effects of said cancer.

The present invention, therefore, contemplates a pharmaceutical composition comprising MCG18 or a derivative thereof or a modulator of *mcg18* expression or MCG18 activity and one or more pharmaceutically acceptable carriers and/or diluents. These components are referred to hereinafter as the "active ingredients". The active ingredients may also include anti-cancer agents or agents which facilitate actions of MCG18.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion or may be in the form of a cream or other form suitable for topical application. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating such as licithin, by the maintenance of the required particle size in the case of dispersion and by the use of superfactants. The preventions of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens,

chlorobutanol, phenol, sorbic acid, thirmerosal and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredient into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze-drying technique which yield a powder of the active ingredient plus any additional desired ingredient from previously sterile-filtered solution thereof.

When the active ingredients are suitably protected they may be orally administered, for example, with an inert diluent or with an assimilable edible carrier, or it may be enclosed in hard or soft shell gelatin capsule, or it may be compressed into tablets, or it may be incorporated directly with the food of the diet. For oral therapeutic administration, the active compound may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Such compositions and preparations should contain at least 1% by weight of active compound. The percentage of the compositions and preparations may, of course, be varied and may conveniently be between about 5 to about 80% of the weight of the unit. The amount of active compound in such therapeutically useful compositions in such that a suitable dosage will be obtained. Preferred compositions or preparations according to the present invention are prepared so that an oral dosage unit form contains between about 0.1 μg and 2000 mg of active compound.

The tablets, troches, pills, capsules and the like may also contain the components as listed hereafter: A binder such as gum, acacia, corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, potato starch, alginic acid and the like; a lubricant such as magnesium stearate; and a sweetening agent such a sucrose, lactose or

saccharin may be added or a flavouring agent such as peppermint, oil of wintergreen, or cherry flavouring. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with shellac, sugar or both. A syrup or elixir may contain the active compound, sucrose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavouring such as cherry or orange flavour. Of course, any material used in preparing any dosage unit form should be pharmaceutically pure and substantially non-toxic in the amounts employed. In addition, the active compound(s) may be incorporated into sustained-release preparations and formulations.

The present invention also extends to forms suitable for topical application such as creams, lotions and gels.

Pharmaceutically acceptable carriers and/or diluents include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, use thereof in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the novel dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active material and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active material for the treatment of disease in living subjects having a diseased condition in which bodily health is impaired as herein disclosed in detail.

The principal active ingredient is compounded for convenient and effective administration in effective amounts with a suitable pharmaceutically acceptable carrier in dosage unit form as hereinbefore disclosed. A unit dosage form can, for example, contain the principal active compound in amounts ranging from 0.5 µg to about 2000 mg. Expressed in proportions, the active compound is generally present in from about 0.5 µg to about 2000 mg/ml of carrier. In the case of compositions containing supplementary active ingredients, the dosages are determined by reference to the usual dose and manner of administration of the said ingredients.

The pharmaceutical composition may also comprise genetic molecules such as a vector capable of transfecting target cells where the vector carries a nucleic acid molecule capable of modulating *mcg18* expression or MCG18 activity. The vector may, for example, be a viral vector.

As stated above, the present invention further contemplates a range of derivatives of MCG18. Derivatives include fragments, parts, portions, mutants, homologues and analogues of the MCG18 polypeptide and corresponding genetic sequence. Derivatives also include single or multiple amino acid substitutions, deletions and/or additions to MCG18 or single or multiple nucleotide substitutions, deletions and/or additions to the genetic sequence encoding MCG18. "Additions" to amino acid sequences or nucleotide sequences include fusions with other peptides, polypeptides or proteins or fusions to nucleotide sequences. Reference herein to "MCG18" includes reference to all derivatives thereof including functional derivatives or MCG18 immunologically interactive derivatives.

Analogues of MCG18 contemplated herein include, but are not limited to, modification to side chains, incorporating of unnatural amino acids and/or their derivatives during peptide, polypeptide or protein synthesis and the use of crosslinkers and other methods which impose conformational constraints on the proteinaceous molecule or their analogues.

Examples of side chain modifications contemplated by the present invention include modifications of amino groups such as by reductive alkylation by reaction with an aldehyde followed by reduction with $NaBH_4$; amidination with methylacetimidate; acylation with acetic anhydride; carbamoylation of amino groups with cyanate; trinitrobenzylation of amino groups

with 2, 4, 6-trinitrobenzene sulphonic acid (TNBS); acylation of amino groups with succinic anhydride and tetrahydrophthalic anhydride; and pyridoxylation of lysine with pyridoxal-5-phosphate followed by reduction with $NaBH_4$.

The guanidine group of arginine residues may be modified by the formation of heterocyclic condensation products with reagents such as 2,3-butanedione, phenylglyoxal and glyoxal.

The carboxyl group may be modified by carbodiimide activation *via* O-acylisourea formation followed by subsequent derivitisation, for example, to a corresponding amide.

Sulphydryl groups may be modified by methods such as carboxymethylation with iodoacetic acid or iodoacetamide; performic acid oxidation to cysteic acid; formation of a mixed disulphides with other thiol compounds; reaction with maleimide, maleic anhydride or other substituted maleimide; formation of mercurial derivatives using 4-chloromercuribenzoate, 4-chloromercuriphenylsulphonic acid, phenylmercury chloride, 2-chloromercuri-4-nitrophenol and other mercurials; carbamoylation with cyanate at alkaline pH.

Tryptophan residues may be modified by, for example, oxidation with N-bromosuccinimide or alkylation of the indole ring with 2-hydroxy-5-nitrobenzyl bromide or sulphenyl halides. Tyrosine residues on the other hand, may be altered by nitration with tetranitromethane to form a 3-nitrotyrosine derivative.

Modification of the imidazole ring of a histidine residue may be accomplished by alkylation with iodoacetic acid derivatives or N-carbethoxylation with diethylpyrocarbonate.

Examples of incorporating unnatural amino acids and derivatives during peptide synthesis include, but are not limited to, use of norleucine, 4-amino butyric acid, 4-amino-3-hydroxy-5-phenylpentanoic acid, 6-aminohexanoic acid, t-butylglycine, norvaline, phenylglycine, ornithine, sarcosine, 4-amino-3-hydroxy-6-methylheptanoic acid, 2-thienyl alanine and/or D-isomers of amino acids. A list of unnatural amino acids, contemplated herein is shown in Table 1.

Crosslinkers can be used, for example, to stabilise 3D conformations, using homo-bifunctional crosslinkers such as the bifunctional imido esters having $(CH_2)_n$ spacer groups with n=1 to n=6, glutaraldehyde, N-hydroxysuccinimide esters and hetero-bifunctional reagents which usually contain an amino-reactive moiety such as N-hydroxysuccinimide and another group specific-reactive moiety such as maleimido or dithio moiety (SH) or carbodiimide (COOH). In addition, peptides can be conformationally constrained by, for example, incorporation of C_α and N_α-methylamino acids, introduction of double bonds between C_α and C_β atoms of amino acids and the formation of cyclic peptides or analogues by introducing covalent bonds such as forming an amide bond between the N and C termini, between two side chains or between a side chain and the N or C terminus.

The present invention further contemplates chemical analogues of MCG18 capable of acting as antagonists or agonists of MCG18 or which can act as functional analogues of MCG18. Chemical analogues may not necessarily be derived from MCG18 but may share certain conformational similarities. Alternatively, chemical analogues may be specifically designed to mimic certain physiochemical properties of MCG18. Chemical analogues may be chemically synthesised or may be detected following, for example, natural product screening.

The identification of MCG18 permits the generation of a range of therapeutic molecules capable of modulating expression of MCG18 or modulating the activity of MCG18. Modulators contemplated by the present invention includes agonists and antagonists of MCG18 expression. Antagonists of MCG18 expression include antisense molecules, ribozymes and co-suppression molecules. Agonists include molecules which increase promoter ability or interfere with negative regulatory mechanisms. Agonists of MCG18 include molecules which overcome any negative regulatory mechanism. Antagonists of MCG18 include antibodies and inhibitor peptide fragments.

TABLE 1

Non-conventional amino acid	Code	Non-conventional amino acid	Code
α-aminobutyric acid	Abu	L-N-methylalanine	Nmala
α-amino-α-methylbutyrate	Mgabu	L-N-methylarginine	Nmarg
aminocyclopropane-carboxylate	Cpro	L-N-methylasparagine	Nmasn
		L-N-methylaspartic acid	Nmasp
aminoisobutyric acid	Aib	L-N-methylcysteine	Nmcys
aminonorbornyl-carboxylate	Norb	L-N-methylglutamine	Nmgln
		L-N-methylglutamic acid	Nmglu
cyclohexylalanine	Chexa	L-N-methylhistidine	Nmhis
cyclopentylalanine	Cpen	L-N-methylisolleucine	Nmile
D-alanine	Dal	L-N-methylleucine	Nmleu
D-arginine	Darg	L-N-methyllysine	Nmlys
D-aspartic acid	Dasp	L-N-methylmethionine	Nmmet
D-cysteine	Dcys	L-N-methylnorleucine	Nmnle
D-glutamine	Dgln	L-N-methylnorvaline	Nmnva
D-glutamic acid	Dglu	L-N-methylornithine	Nmorn
D-histidine	Dhis	L-N-methylphenylalanine	Nmphe
D-isoleucine	Dile	L-N-methylproline	Nmpro
D-leucine	Dleu	L-N-methylserine	Nmser
D-lysine	Dlys	L-N-methylthreonine	Nmthr
D-methionine	Dmet	L-N-methyltryptophan	Nmtrp
D-ornithine	Dorn	L-N-methyltyrosine	Nmtyr
D-phenylalanine	Dphe	L-N-methylvaline	Nmval
D-proline	Dpro	L-N-methylethylglycine	Nmetg
D-serine	Dser	L-N-methyl-t-butylglycine	Nmtbug
D-threonine	Dthr	L-norleucine	Nle
D-tryptophan	Dtrp	L-norvaline	Nva

D-tyrosine	Dtyr	α-methyl-aminoisobutyrate	Maib
D-valine	Dval	α-methyl-γ-aminobutyrate	Mgabu
D-α-methylalanine	Dmala	α-methylcyclohexylalanine	Mchexa
D-α-methylarginine	Dmarg	α-methylcylcopentylalanine	Mcpen
D-α-methylasparagine	Dmasn	α-methyl-α-napthylalanine	Manap
D-α-methylaspartate	Dmasp	α-methylpenicillamine	Mpen
D-α-methylcysteine	Dmcys	N-(4-aminobutyl)glycine	Nglu
D-α-methylglutamine	Dmgln	N-(2-aminoethyl)glycine	Naeg
D-α-methylhistidine	Dmhis	N-(3-aminopropyl)glycine	Norn
D-α-methylisoleucine	Dmile	N-amino-α-methylbutyrate	Nmaabu
D-α-methylleucine	Dmleu	α-napthylalanine	Anap
D-α-methyllysine	Dmlys	N-benzylglycine	Nphe
D-α-methylmethionine	Dmmet	N-(2-carbamylethyl)glycine	Ngln
D-α-methylornithine	Dmorn	N-(carbamylmethyl)glycine	Nasn
D-α-methylphenylalanine	Dmphe	N-(2-carboxyethyl)glycine	Nglu
D-α-methylproline	Dmpro	N-(carboxymethyl)glycine	Nasp
D-α-methylserine	Dmser	N-cyclobutylglycine	Ncbut
D-α-methylthreonine	Dmthr	N-cycloheptylglycine	Nchep
D-α-methyltryptophan	Dmtrp	N-cyclohexylglycine	Nchex
D-α-methyltyrosine	Dmty	N-cyclodecylglycine	Ncdec
D-α-methylvaline	Dmval	N-cylcododecylglycine	Ncdod
D-N-methylalanine	Dnmala	N-cyclooctylglycine	Ncoct
D-N-methylarginine	Dnmarg	N-cyclopropylglycine	Ncpro
D-N-methylasparagine	Dnmasn	N-cycloundecylglycine	Ncund
D-N-methylaspartate	Dnmasp	N-(2,2-diphenylethyl)glycine	Nbhm
D-N-methylcysteine	Dnmcys	N-(3,3-diphenylpropyl)glycine	Nbhe
D-N-methylglutamine	Dnmgln	N-(3-guanidinopropyl)glycine	Narg
D-N-methylglutamate	Dnmglu	N-(1-hydroxyethyl)glycine	Nthr
D-N-methylhistidine	Dnmhis	N-(hydroxyethyl))glycine	Nser
D-N-methylisoleucine	Dnmile	N-(imidazolylethyl))glycine	Nhis
D-N-methylleucine	Dnmleu	N-(3-indolylyethyl)glycine	Nhtrp

	D-N-methyllysine	Dnmlys	N-methyl-γ-aminobutyrate	Nmgabu
	N-methylcyclohexylalanine	Nmchexa	D-N-methylmethionine	Dnmmet
	D-N-methylornithine	Dnmorn	N-methylcyclopentylalanine	Nmcpen
	N-methylglycine	Nala	D-N-methylphenylalanine	Dnmphe
	N-methylaminoisobutyrate	Nmaib	D-N-methylproline	Dnmpro
	N-(1-methylpropyl)glycine	Nile	D-N-methylserine	Dnmser
	N-(2-methylpropyl)glycine	Nleu	D-N-methylthreonine	Dnmthr
	D-N-methyltryptophan	Dnmtrp	N-(1-methylethyl)glycine	Nval
	D-N-methyltyrosine	Dnmtyr	N-methyla-napthylalanine	Nmanap
	D-N-methylvaline	Dnmval	N-methylpenicillamine	Nmpen
	γ-aminobutyric acid	Gabu	N-(*p*-hydroxyphenyl)glycine	Nhtyr
	L-*t*-butylglycine	Tbug	N-(thiomethyl)glycine	Ncys
	L-ethylglycine	Etg	penicillamine	Pen
	L-homophenylalanine	Hphe	L-α-methylalanine	Mala
	L-α-methylarginine	Marg	L-α-methylasparagine	Masn
	L-α-methylaspartate	Masp	L-α-methyl-*t*-butylglycine	Mtbug
	L-α-methylcysteine	Mcys	L-methylethylglycine	Metg
	L-α-methylglutamine	Mgln	L-α-methylglutamate	Mglu
	L-α-methylhistidine	Mhis	L-α-methylhomophenylalanine	Mhphe
	L-α-methylisoleucine	Mile	N-(2-methylthioethyl)glycine	Nmet
	L-α-methylleucine	Mleu	L-α-methyllysine	Mlys
	L-α-methylmethionine	Mmet	L-α-methylnorleucine	Mnle
	L-α-methylnorvaline	Mnva	L-α-methylornithine	Morn
	L-α-methylphenylalanine	Mphe	L-α-methylproline	Mpro
	L-α-methylserine	Mser	L-α-methylthreonine	Mthr
	L-α-methyltryptophan	Mtrp	L-α-methyltyrosine	Mtyr

L-α-methylvaline	Mval	L-N-methylhomophenylalanine	Nmhphe
N-(N-(2,2-diphenylethyl) carbamylmethyl)glycine	Nnbhm	N-(N-(3,3-diphenylpropyl) carbamylmethyl)glycine	Nnbhe
1-carboxy-1-(2,2-diphenyl-ethylamino)cyclopropane	Nmbc		

These types of modifications may be important to stabilise MCG18 if administered to an individual or for use as a diagnostic reagent.

Other derivatives contemplated by the present invention include a range of glycosylation variants from a completely unglycosylated molecule to a modified glycosylated molecule. Altered glycosylation patterns may result from expression of recombinant molecules in different host cells.

Another embodiment of the present invention contemplates a method for modulating expression of MCG18 in a human, said method comprising contacting the *mcg18* gene encoding MCG18 with an effective amount of a modulator of *mcg18* expression for a time and under conditions sufficient to up-regulate or down-regulate or otherwise modulate expression of *mcg18*. For example, a nucleic acid molecule encoding MCG18 or a derivative thereof may be introduced into a cell to facilitate protection of that cell from becoming cancerous.

Another aspect of the present invention contemplates a method of modulating activity of MCG18 in a human, said method comprising administering to said mammal a modulating effective amount of a molecule for a time and under conditions sufficient to increase or decrease MCG18 activity. The molecule may be a proteinaceous molecule or a chemical entity and may also be a derivative of MCG18 or a chemical analogue or truncation mutant of MCG18.

The present invention is further described with reference to the following non-limiting

Figures and Examples.

In the Figures:

Figure 1 is a representation of the nucleotide sequence and corresponding amino acid sequence of *mcg18*.

Figure 2 is a representation showing that MCG18 has partial homology to *E. coli* DnaJ.

Figure 3 is a representation showing that MCG18 has homology to a *Caenorhabitis elegans* protein.

Figure 4 is a representation showing that MCG18 has homology to a *Saccharomyces pombe* protein.

Figure 5 is a representation showing homology of MCG18 to a *Drosophila virilis* protein.

Figure 6 is a representation showing homology of MCG18 to human DnaJ proteins HDJ-2/HSDJ, HDJ-1/HSP40 and HSJ1.

EXAMPLE 1

A human gene was identified from chromosome 11q13 that encodes a new member of the DnaJ family of proteins (designated MCG18). This gene (*mcg18)* is expressed as an ~1.4kb mRNA and is predicted to encode a 241 amino acid product (Fig. 1).

EXAMPLE 2

MCG18 has partial homology to *E. coli* dnaJ and other human DnaJ family members in that it contains the J domain (Fig. 2).

EXAMPLE 3

MCG18 has greatest homology to functionally undefined proteins from *C. elegans* (Fig. 3) and *S. pombe* (Fig. 4) that also feature the J domain but maintain sequence similarity through the central and C-terminal regions of the proteins.

EXAMPLE 4

The J domain is proposed to mediate interaction with heat shock 70 protein (Hsp70) and consist of some 70 amino acids, frequently located at the N-terminus of the protein. One of these proteins, tumorous imaginal discs (Tid58) from *Drosophila virillis* (Figure 5) functions as a tumour suppressor.

EXAMPLE 5

A comparison of homology between MCG18 and human DnaJ proteins HDJ-2/H3DJ, HDJ-1/HSP40 and HSJ1 is shown in Figure 6.

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: The Council of The Queensland Institute of Medical Research

(ii) TITLE OF INVENTION: A NOVEL GENE AND USES THEREFOR

(iii) NUMBER OF SEQUENCES: 2

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: DAVIES COLLISON CAVE
(B) STREET: 1 LITTLE COLLINS STREET
(C) CITY: MELBOURNE
(D) STATE: VICTORIA
(E) COUNTRY: AUSTRALIA
(F) ZIP: 3000

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: AUSTRALIAN PROVISIONAL
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: HUGHES, DR E JOHN L
(C) REFERENCE/DOCKET NUMBER: EJH/AF

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: +61 3 9254 2777
(B) TELEFAX: +61 3 9254 2770
(C) TELEX: AA 31787

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 832 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 11..733

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
GCCCGCCGCC ATG CCG CCC TTA CTG CCC CTG CGC CTG TGC CGG CTG TGG          49
           Met Pro Pro Leu Leu Pro Leu Arg Leu Cys Arg Leu Trp
             1               5                  10

CCC CGC AAC CCT CCC TCC CGG CTC CTC GGA GCG GCC GCC GGG CAG CGG          97
Pro Arg Asn Pro Pro Ser Arg Leu Leu Gly Ala Ala Ala Gly Gln Arg
     15                  20                  25

TCC AGA CCC AGT ACT TAT TAT GAA CTG TTG GGG GTG CAT CCT GGT GCC         145
Ser Arg Pro Ser Thr Tyr Tyr Glu Leu Leu Gly Val His Pro Gly Ala
 30                  35                  40                  45

AGC ACT GAG GAA GTT AAA CGA GCT TTC TTC TCC AAG TCC AAA GAG CTG         193
Ser Thr Glu Glu Val Lys Arg Ala Phe Phe Ser Lys Ser Lys Glu Leu
                 50                  55                  60

CAC CCA GAC CGG GAC CCT GGG AAC CCA AGC CTG CAC AGC CGC TTT GTG         241
His Pro Asp Arg Asp Pro Gly Asn Pro Ser Leu His Ser Arg Phe Val
           65                  70                  75

GAG CTG AGC GAG GCA TAC CGT GTG CTC AGC CGT GAG CAG AGC CGC CGC         289
Glu Leu Ser Glu Ala Tyr Arg Val Leu Ser Arg Glu Gln Ser Arg Arg
       80                  85                  90

AGC TAT GAT GAC CAG CTC CGC TCA GGT AGT CCC CCA AAG TCT CCA CGA         337
Ser Tyr Asp Asp Gln Leu Arg Ser Gly Ser Pro Pro Lys Ser Pro Arg
     95                  100                 105

ACC ACA GTC CAT GAC AAG TCT GCC CAC CAA ACA CAC AGC TCC TGG ACA         385
Thr Thr Val His Asp Lys Ser Ala His Gln Thr His Ser Ser Trp Thr
110                 115                 120                 125

CCC CCC AAC GCA CAG TAC TGG TCC CAG TTT CAC AGC GTG AGG CCA CAG         433
Pro Pro Asn Ala Gln Tyr Trp Ser Gln Phe His Ser Val Arg Pro Gln
                130                 135                 140

GGG CCC CAG TTG AGG CAG CAG CAA CAC AAA CAA AAC AAA CAA GTG CTG         481
Gly Pro Gln Leu Arg Gln Gln Gln His Lys Gln Asn Lys Gln Val Leu
           145                 150                 155

GGG TAC TGC CTC CTC CTC ATG CTG GCG GGC ATG GGC CTG CAC TAC ATT         529
Gly Tyr Cys Leu Leu Leu Met Leu Ala Gly Met Gly Leu His Tyr Ile
       160                 165                 170

GCC TTC AGG AAG GTG AAG CAG ATG CAC CTT AAC TTC ATG GAT GAA AAG         577
Ala Phe Arg Lys Val Lys Gln Met His Leu Asn Phe Met Asp Glu Lys
     175                 180                 185

GAT CGG ATC ATC ACA GCC TTC TAC AAC GAA GCC CGG GCA CGG GCC AGG         625
Asp Arg Ile Ile Thr Ala Phe Tyr Asn Glu Ala Arg Ala Arg Ala Arg
190                 195                 200                 205

GCC AAC AGA GGC ATC CTT CAG CAG GAG CGA CAA CGG CTA GGG CAG CGG         673
Ala Asn Arg Gly Ile Leu Gln Gln Glu Arg Gln Arg Leu Gly Gln Arg
                210                 215                 220

CAG CCG CCA CCA TCC GAG CCA ACC CAA GGC CCC GAG ATC GTG CCC CGG         721
```

```
Gln Pro Pro Pro Ser Glu Pro Thr Gln Gly Pro Glu Ile Val Pro Arg
225                 230                 235

GGC GCC GGC CCC TGA GGGGCTC ACCTGGATGG GGCCTGCAGT GCGTTCCCGC          773
Gly Ala Gly Pro   *
240

TTTGCTTCCT TCCCTGGACG GCCCGCTCCC CGAAACGCGC GCAATAAAGT GATTCGCAG      832
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 241 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Met Pro Pro Leu Leu Pro Leu Arg Leu Cys Arg Leu Trp Pro Arg Asn
 1               5                  10                  15

Pro Pro Ser Arg Leu Leu Gly Ala Ala Ala Gly Gln Arg Ser Arg Pro
            20                  25                  30

Ser Thr Tyr Tyr Glu Leu Leu Gly Val His Pro Gly Ala Ser Thr Glu
        35                  40                  45

Glu Val Lys Arg Ala Phe Phe Ser Lys Ser Lys Glu Leu His Pro Asp
    50                  55                  60

Arg Asp Pro Gly Asn Pro Ser Leu His Ser Arg Phe Val Glu Leu Ser
65                  70                  75                  80

Glu Ala Tyr Arg Val Leu Ser Arg Glu Gln Ser Arg Arg Ser Tyr Asp
                85                  90                  95

Asp Gln Leu Arg Ser Gly Ser Pro Pro Lys Ser Pro Arg Thr Thr Val
            100                 105                 110

His Asp Lys Ser Ala His Gln Thr His Ser Ser Trp Thr Pro Pro Asn
        115                 120                 125

Ala Gln Tyr Trp Ser Gln Phe His Ser Val Arg Pro Gln Gly Pro Gln
    130                 135                 140

Leu Arg Gln Gln Gln His Lys Gln Asn Lys Gln Val Leu Gly Tyr Cys
145                 150                 155                 160

Leu Leu Leu Met Leu Ala Gly Met Gly Leu His Tyr Ile Ala Phe Arg
                165                 170                 175

Lys Val Lys Gln Met His Leu Asn Phe Met Asp Glu Lys Asp Arg Ile
            180                 185                 190

Ile Thr Ala Phe Tyr Asn Glu Ala Arg Ala Arg Ala Arg Ala Asn Arg
        195                 200                 205

Gly Ile Leu Gln Gln Glu Arg Gln Arg Leu Gly Gln Arg Gln Pro Pro
    210                 215                 220

Pro Ser Glu Pro Thr Gln Gly Pro Glu Ile Val Pro Arg Gly Ala Gly
225                 230                 235                 240
```

Pro

DATED this 23rd day of May 1997

The Council of The Queensland Institute of Medical Research

By DAVIES COLLISON CAVE

Patent Attorneys for the Applicants

FIGURE 1

```
GCCCGCCGCC ATG CCG CCC TTA CTG CCC CTG CGC CTG TGC CGG CTG TGG          49
           Met Pro Pro Leu Leu Pro Leu Arg Leu Cys Arg Leu Trp
             1               5                  10

CCC CGC AAC CCT CCC TCC CGG CTC CTC GGA GCG GCC GCC GGG CAG CGG          97
Pro Arg Asn Pro Pro Ser Arg Leu Leu Gly Ala Ala Ala Gly Gln Arg
     15                  20                  25

TCC AGA CCC AGT ACT TAT TAT GAA CTG TTG GGG GTG CAT CCT GGT GCC         145
Ser Arg Pro Ser Thr Tyr Tyr Glu Leu Leu Gly Val His Pro Gly Ala
 30                  35                  40                  45

AGC ACT GAG GAA GTT AAA CGA GCT TTC TTC TCC AAG TCC AAA GAG CTG         193
Ser Thr Glu Glu Val Lys Arg Ala Phe Phe Ser Lys Ser Lys Glu Leu
                 50                  55                  60

CAC CCA GAC CGG GAC CCT GGG AAC CCA AGC CTG CAC AGC CGC TTT GTG         241
His Pro Asp Arg Asp Pro Gly Asn Pro Ser Leu His Ser Arg Phe Val
           65                  70                  75

GAG CTG AGC GAG GCA TAC CGT GTG CTC AGC CGT GAG CAG AGC CGC CGC         289
Glu Leu Ser Glu Ala Tyr Arg Val Leu Ser Arg Glu Gln Ser Arg Arg
         80                  85                  90

AGC TAT GAT GAC CAG CTC CGC TCA GGT AGT CCC CCA AAG TCT CCA CGA         337
Ser Tyr Asp Asp Gln Leu Arg Ser Gly Ser Pro Pro Lys Ser Pro Arg
     95                  100                 105

ACC ACA GTC CAT GAC AAG TCT GCC CAC CAA ACA CAC AGC TCC TGG ACA         385
Thr Thr Val His Asp Lys Ser Ala His Gln Thr His Ser Ser Trp Thr
110                 115                 120                 125

CCC CCC AAC GCA CAG TAC TGG TCC CAG TTT CAC AGC GTG AGG CCA CAG         433
Pro Pro Asn Ala Gln Tyr Trp Ser Gln Phe His Ser Val Arg Pro Gln
                130                 135                 140

GGG CCC CAG TTG AGG CAG CAG CAA CAC AAA CAA AAC AAA CAA GTG CTG         481
Gly Pro Gln Leu Arg Gln Gln Gln His Lys Gln Asn Lys Gln Val Leu
           145                 150                 155

GGG TAC TGC CTC CTC CTC ATG CTG GCG GGC ATG GGC CTG CAC TAC ATT         529
Gly Tyr Cys Leu Leu Leu Met Leu Ala Gly Met Gly Leu His Tyr Ile
         160                 165                 170

GCC TTC AGG AAG GTG AAG CAG ATG CAC CTT AAC TTC ATG GAT GAA AAG         577
Ala Phe Arg Lys Val Lys Gln Met His Leu Asn Phe Met Asp Glu Lys
     175                 180                 185

GAT CGG ATC ATC ACA GCC TTC TAC AAC GAA GCC CGG GCA CGG GCC AGG         625
```

Figure 1 (continued)

```
Asp Arg Ile Ile Thr Ala Phe Tyr Asn Glu Ala Arg Ala Arg Ala Arg
190                 195                 200                 205

GCC AAC AGA GGC ATC CTT CAG CAG GAG CGA CAA CGG CTA GGG CAG CGG        673
Ala Asn Arg Gly Ile Leu Gln Gln Glu Arg Gln Arg Leu Gly Gln Arg
                210                 215                 220

CAG CCG CCA CCA TCC GAG CCA ACC CAA GGC CCC GAG ATC GTG CCC CGG        721
Gln Pro Pro Pro Ser Glu Pro Thr Gln Gly Pro Glu Ile Val Pro Arg
            225                 230                 235

GGC GCC GGC CCC TGA GGGGCTC ACCTGGATGG GGCCTGCAGT GCGTTCCCGC           773
Gly Ala Gly Pro   *
        240

TTTGCTTCCT TCCCTGGACG GCCCGCTCCC CGAAACGCGC GCAATAAAGT GATTCGCAG       832
```

Figure 2

```
>sp|P08622|DNAJ_ECOLI DNAJ PROTEIN >pir||HHECDJ heat shock protein dnaJ -
            Escherichia coli >gi|145769 (M12565) heat shock protein dnaJ
            [Escherichia coli] >gi|216441 (D10483) dnaJ protein [Escherichia
            coli]
            Length = 376

 Score = 138 (63.7 bits), Expect = 1.2e-10, P = 1.2e-10
 Identities = 25/62 (40%), Positives = 39/62 (62%)

Query:    35 YYELLGVHPGASTEEVKRAFFSKSKELHPDRDPGNPSLHSRFVELSEAYRVLSREQSRRS 94
             YYE+LGV   A   E+++A+   + + HPDR+ G+    ++F E+ EAY VL+  Q R +
Sbjct:     6 YYEILGVSKTAEEREIRKAYKRLAMKYHPDRNQGDKEAEAKFKEIKEAYEVLTDSQKRAA 65

Query:    95 YD 96
             YD
Sbjct:    66 YD 67
```

Figure 3

```
>gi|1703590 (U80439) contains similarity to a DNAJ-like domain [Caenorhabditis
            elegans]
            Length = 345

 Score = 98 (45.2 bits), Expect = 5.2e-12, Sum P(3) = 5.2e-12
 Identities = 17/37 (45%), Positives = 28/37 (75%)

Query:     28 QRSRPSTYYELLGVHPGASTEEVKRAFFSKSKELHPD 64
              ++ R  T+YE+LGV   A+  E+K AF+++SK++HPD
Sbjct:     22 KKIRQRTHYEVLGVESTATLSEIKSAFYAQSKKVHPD 58

 Score = 74 (34.1 bits), Expect = 5.2e-12, Sum P(3) = 5.2e-12
 Identities = 17/32 (53%), Positives = 19/32 (59%)

Query:     71 SLHSRFVELSEAYRVLSREQSRRSYDDQLRSG 102
              S  + F+EL  AY VL R   RR YD QLR G
Sbjct:     64 SATASFLELKNAYDVLRRPADRRLYDYQLRGG 95

 Score = 39 (18.0 bits), Expect = 5.2e-12, Sum P(3) = 5.2e-12
 Identities = 10/42 (23%), Positives = 19/42 (45%)

Query:    162 LLMLAGMGLHYIAFRKVKQMHLNFMDEKDRIITAFYNEARAR 203
              L+++AG    Y+      Q  L+ + ++D I   F  +   R
Sbjct:    158 LVLVAGYNGGYLYLLAYNQKQLDKLIDEDEIAKCFLRQKEFR 199



>gnl|PID|e281266 (Z81030) C01G10.12 [Caenorhabditis elegans]
            Length = 191

 Score = 96 (44.3 bits), Expect = 1.8e-09, Sum P(3) = 1.8e-09
 Identities = 17/41 (41%), Positives = 27/41 (65%)

Query:     35 YYELLGVHPGASTEEVKRAFFSKSKELHPDRDPGNPSLHSR 75
              YYE++GV   A+ +E++ AF  K+K+LHPD+   +    SR
Sbjct:     19 YYEIIGVSASATRQEIRDAFLKKTKQLHPDQSRKSSKSDSR 59

 Score = 54 (24.9 bits), Expect = 1.8e-09, Sum P(3) = 1.8e-09
 Identities = 10/22 (45%), Positives = 15/22 (68%)

Query:     75 RFVELSEAYRVLSRE[illegible]
              +F+ + EAY VL  E+ F+ YD
Sbjct:     71 QFMLVKEAYDVLRNEERKKEYD 92

 Score = 35 (16.1 bits), Expect = 1.8e-09, Sum P(3) = 1.8e-09
 Identities = 9/44 (20%), Positives = 22/44 (50%)

Query:    141 QGPQLRQQQHKQNKQVLGYCLLLMLAGMGLHYIAFRKVKQMHLN 184
              + P+    + KQ  ++L      ++A +G + +  RK++   L+
Sbjct:    145 RNPEDEYLREKQKNRMLAVLAATVMALIGANIVYIRKLQADRLS 188
```

Figure 4

```
>sp|Q10209|YAY1_SCHPO HYPOTHETICAL 44.8 KD PROTEIN C4H3.01 IN CHROMOSOME I
           >gi|1184014 (Z69380) unknown [Schizosaccharomyces pombe]
           Length = 392

 Score = 84 (38.8 bits), Expect = 4.1e-08, Sum P(3) = 4.1e-08
 Identities = 13/36 (36%), Positives = 25/36 (69%)

Query:    35 YYELLGVHPGASTEEVKRAFFSKSKELHPDRDPGNP 70
             YY+LLG+   A+  ++K+A+   + + HPD++P +P
Sbjct:     9 YYDLLGISTDATAVDIKKAYRKLAVKYHPDKNPDDP 44

 Score = 64 (29.5 bits), Expect = 4.1e-08, Sum P(3) = 4.1e-08
 Identities = 14/40 (35%), Positives = 23/40 (57%)

Query:    75 RFVELSEAYRVLSREQSRRSYDDQLRSGSPPKSPRTTVHD 114
             +F ++SEAY+VL  E+ R  YD   +  + P+   T  +D
Sbjct:    50 KFQKISEAYQVLGDEKLRSQYDQFGKEKAVPEQGFTDAYD 89

 Score = 37 (17.1 bits), Expect = 4.1e-08, Sum P(3) = 4.1e-08
 Identities = 9/29 (31%), Positives = 15/29 (51%)

Query:   190 DRIITAFYNEARARARANRGILQQERQRL 218
             DR   A   E  A A+  + +++  RQR+
Sbjct:   149 DRKKNAQIREREALAKREQEMIEDRRQRI 177

 Score = 33 (15.2 bits), Expect = 0.00081, Sum P(3) = 0.00081
 Identities = 8/19 (42%), Positives = 11/19 (57%)

Query:   140 PQGPQLRQQQHKQNKQVLG 158
             PQG   + Q+  +  QVLG
Sbjct:    44 PQGASEKFQKISEAYQVLG 62
```

Figure 5

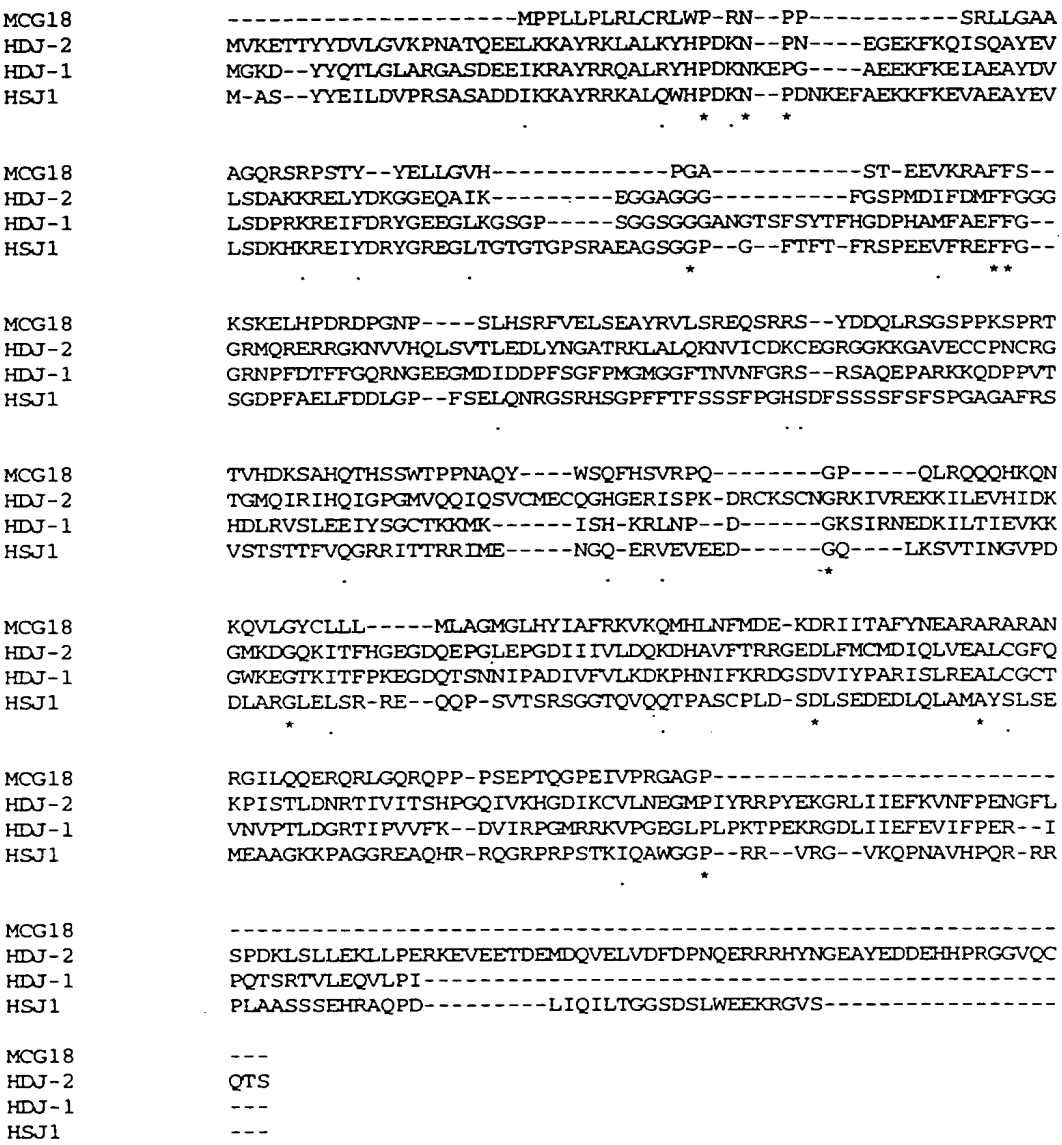
```
>gnl|PID|e253406 (X77635) tumorous imaginal discs [Drosophila virilis]
           >gnl|PID|e263866 (Y07700) Tid58 protein [Drosophila virilis]
           Length = 529

 Score = 153 (70.6 bits), Expect = 9.7e-13, P = 9.7e-13
 Identities = 27/71 (38%), Positives = 44/71 (61%)

Query:    26 AGQRSRPSTYYELLGVHPGASTEEVKRAFFSKSKELHPDRDPGNPSLHSRFVELSEAYRV 85
             +  R +   YY  LGV   A+ +++K+A++  +K+ HPD +  +P    +F ++SEAY V
Sbjct:    72 SSSRMQAKDYYATLGVAKNANAKDIKKAYYELAKKYHPDTNKDDPDASKKFQDVSEAYEV 131

Query:    86 LSREQSRRSYD 96
             LS +Q RR YD
Sbjct:   132 LSDDQKRREYD 142
```

Figure 6



* = amino acid identity in all 4 proteins

. = conservative substitution